(54) Title: SEQUENCE SPECIFIC DNA BINDING DRUGS

(57) Abstract

A sequence specific DNA binding molecule which comprises an oligonucleotide conjugate formed by the covalent attachment of a DNA binding drug to a triplex forming oligonucleotide and a method of modulating gene expression comprising administering to a mammal in need thereof said sequence specific binding molecule.

SEQUENCE SPECIFIC DNA BINDING DRUGS

The present invention relates to a novel group of compounds and their method of use. The compounds of the instant invention use triplex forming oligonucleotides to target DNA binding drugs, such as acridine or mithramycin, to specific protein binding sequences in the promoters of various genes. The oligonucleotide conjugates of the instant invention can specifically bind to protein binding regions in the promoters of genes, thus preventing protein binding and transcription initiation. In a preferred embodiment, the combination of the sequence specificity, but low affinity of triplex formation, with the high affinity but low sequence specificity of the DNA binding drug acridine, results in an acridine-oligonucleotide conjugate which binds DNA in a sequence specific manner.

BACKGROUND OF THE INVENTION

It has been known, since the early sixties, that under certain conditions DNA can form triple stranded DNA structures known as triplex DNA. Considerable speculation has been given to the potential use of this sequence specific interaction to target therapeutic agents, particularly since the observation that the target sequences do not need to be exclusively polypurine and polypyrimidine. However, because of the relatively low affinity of triplex binding oligonucleotides and because of uncertainty of the best target sequences for triplex formation, this approach has not been effective. The instant compounds, such as the instant acridine-oligonucleotide conjugate, represent an effort to solve both of these problems.

Based on the observation of leukemic cell differentiation in a single patient with blast phase chronic myelogenous leukemia, the inventor has studied the mechanism by which DNA binding drugs alter gene expression. Over the past several years, the inventor has demonstrated that the G-C specific DNA binding compound, mithramycin, inhibits RNA synthesis by preventing regulatory protein binding to G-C rich promoters. However, currently available DNA binding drugs are inadequately sequence specific to effectively inhibit expression of single genes.

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Based on earlier work with mithramycin, the inventor has found
that important protein binding sequences in the c-myc and DHFR
promoters can form triplex DNA structures. It also has been shown
that triplex formation by protein binding sequences in the c-myc
5   and DHFR promoters prevents protein binding in these sequences.
However, the affinity of binding is relatively low and they have
not been able to demonstrate altered transcriptional activity of
either of these genes when whole cells are exposed to high
concentrations of triplex forming oligonucleotides. Thus, the
10   inventor has discovered that the synthetic attachment of acridine
to the oligonucleotide was a means of increasing the binding
affinity which has indeed lead to intracellular activity.

SUMMARY OF THE INVENTION

15   The invention relates to sequence specific DNA binding
compounds useful for the modulation of gene expression due to their
ability to bind specifically to the protein binding regions in gene
promoters and thus, prevent regulatory protein binding and
transcription initiation. The sequence specific compounds of the
20   instant   invention   are   conjugates   of   triplex   forming
oligonucleotides and DNA binding drugs. The compounds are produced
by covalently linking a DNA binding drug, such as acridine, to a
triplex forming oligonucleotide, to form, for example, an acridine-
oligonucleotide conjugate. Advantageously, the instant conjugates
25   possess   excellent   specificity   and   affinity   and   demonstrate
intracellular activity when introduced to whole cells.

DESCRIPTION OF THE DRAWINGS

Figure 1 represents triplex formation by c-myc promoter
30   targeted acridine-oligonucleotide conjugate. The c-myc promoter
(-115 to 142 bp) triplex forming oligonucleotide (G-rich oligo)
was covalently attached to the intercalating agent acridine. The
modified compound was then added to the complement of the attached
oligonucleotide, or to duplex DNA formed by annealing the G-rich
35   oligonucleotide with its complement. Set (A) shows duplex and
triplex formation by the G-rich (G27) conjugate but not by a
conjugate which contains a two base substitution. Set (B) shows
duplex, but not triplex formation by the C-rich (C27) conjugate.
This figure demonstrates that the targeted acridine compound can

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form duplex molecules distinguishable from the G-rich/C-rich duplex
(Lanes 2 and 3). It also demonstrates that the targeted acridine
can form triplex structures when added to the preformed
oligonucleotide duplex DNA. Subsequent experiments have

5   demonstrated that the addition of targeted compound simultaneously
with the oligonucleotides also results in triplex formation.

Figure 2 represents sequence specificity of triplex formation
by c-myc targeted acridine-oligonucleotide conjugate. The G-rich
c-myc triplex forming oligonucleotide (complementary to sequence

10   -115 to -142) was covalently attached to acridine with a 6
methylene linker as described in the Examples. The targeted
compound was reacted with the 87 bp c-myc promoter triplex forming
fragment under triplex forming conditions as follows. The
resultant complex was digested with DNAse I and the products

15   analyzed on an 8%, 8M urea polyacrylamide DNA sequencing gel.
Oligonucleotide alone was used as a control. The C-rich
oligonucleotide and the C-rich conjugate did not form triplex. The
resultant autoradiogram, which is shown here, demonstrates triplex
formation with the target sequence by both the G-rich

20   oligonucleotide and the G-rich oligonucleotide-acridine conjugate.
Control Maxam Gilbert reactions were used to localize the sequence
specificity of triplex formation. Analysis of binding to slightly
mismatched sequences also failed to demonstrate triplex formation.

Figure 3 represents inhibition of protein binding of the c-

25   myc 5' flanking region by triplex forming acridine conjugate. The
87 bp c-myc 5' fragment containing the sequence -115 to -142 was
endlabelled and incubated in a HeLa cell nuclear extract. The
effect of the c-myc targeted acridine-oligonucleotide conjugate was
determined by gel shift analysis. It is clear from the figure

30   (individual lanes are labelled) that the G-rich oligonucleotide and
the G-rich oligonucleotide-acridine conjugate, but not the C-rich
oligonucleotide conjugate, prevent protein binding to this
fragment. A mismatched oligonucleotide and its conjugate (3 bp
mismatch) also did not form triplex or inhibit protein binding to

35   this sequence. There are three DNA-protein complexes only two of
which are inhibited by the acridine conjugate. This suggests that
the third protein complex involves DNA sequences outside the target
sequence. Preliminary footprint analysis has confirmed this
conclusion.

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Figure 4 represents inhibition of HL-60 cellular proliferation
by the anti-c-myc oligonucleotide-acridine conjugate. HL-60 cells
(5×10^5 cells/ml) were treated with the oligonucleotide-conjugate
at a concentration of 5 um for 72 hours. Cell counts revealed a
5   significant inhibition of cellular proliferation by the conjugate.
Controls included oligonucleotide and phosphorylated
oligonucleotide alone, neither of which had a significant effect
on proliferation. The data shown is the average of three
experiments with a variability of <5%.

10   Figure 5 represents inhibition of DNA synthesis of HL-60 cells
by the anti-c-myc acridine conjugate. HL-60 cells (5×10^5
cells/ml) were treated with the drug conjugate at a concentration
of 5 um for 72 hours. Measurement of thymidine incorporation
revealed a significant inhibition of DNA synthesis which correlated
15   directly with the effect of this compound on cellular
proliferation. Controls included oligonucleotide alone, and
acridine alone. Comparison of the effect of 5.0 and 2.5 um
oligonucleotide-acridine conjugate reveal concentration dependence.

Figure 6 represents inhibition of HL-60 c-myc expression by
20   the anti-c-myc acridine conjugate. HL-60 cells (5×10^5 cells/ml)
were treated with the drug conjugate at a concentration of 5 um for
48 hours. Total cellular RNA was extracted and c-myc expression
was analyzed by dot hybridization. There was a dramatic inhibition
of c-myc expression (90%) by 24 hours with the triplex forming
25   conjugate. On the other hand, the cells treated with the
nontriplex forming (complementary) oligonucleotide conjugate, or
the conjugate formed with the triplex forming oligonucleotide with
three base substitutions did not show inhibition of c-myc
expression or cellular proliferation. Acridine at a concentration
30   of 2 or 5 um did not inhibit c-myc expression. Control experiments
showed no effect of this treatment on the level of mRNA specific
for gamma-actin, DHFR, or 18S rRNA.

                    DETAILED DESCRIPTION OF THE INVENTION
35   The instant compounds are conjugates of triplex forming
oligonucleotides and DNA binding drugs. The binding drugs are
covalently linked to appropriate oligonucleotides to form a
conjugate. The resulting conjugates bind specifically to the
protein binding sequence in the target gene to prevent regulatory

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protein binding and transcription initiation.   The inhibition of
gene expression results because of the triplex formation and DNA
binding drug prevent regulatory protein binding and transcription.

5   The conjugates of the instant invention are prepared by first
modifying the DNA binding drug or intercalating agent with a
linker.   Among the DNA binding drugs there may be mentioned
Actinomycin D, Doxorubicin, Daunorubicin, Mithramycin, Mitomycin
C, Bleomycin, Distamycin, Netropsin and Lexitropsin.   The linker
molecules are typically straight chain lower alkyl alcohols which
10   are suitable for covalent attachment to the 3' or 5' end of the
oligonucleotide.   Particularly suitable linkers have from 3 to 7
methylene groups in the alkyl chain such as C_{3-7} amino alcohols
represented by the formula $H_2N(CH_2)_nOH$, wherein n=3 to 7.   It should
be recognized that somewhat longer alkyl chains would be suitable
15   for this process.   Once the DNA binding drug is modified with the
linker, it can then be covalently attached to the 5'phosphate group
of the oligonucleotide by a condensation reaction.   Similarly,
groups suitable for the condensation to the 3' hydroxyl group of
the oligonucleotide can be readily substituted for the hydroxyl
20   group on the end of the alkyl chain of the linker.   Suitable groups
for the covalent attachment to the 5' phosphate or 3' hydroxyl, as
well as the preparation of the linker are well within the skill in
the art using conventional synthetic techniques.

Once the linker molecule is formed, it can then be conjugated
25   to any appropriate oligonucleotide.   The choice of target sequences
for the conjugate synthesis of the instant invention is dependent
on both the absolute sequence and localization of the sequence
relative to the transcription start site(s) of the relevant genes.
Polypurine-polypyrimidine sequences are disproportionately common
30   in the 5' flanking regions of eukaryotic genes.   Many of these
sequences are protein binding sequences which appear to play an
important role in the regulation of gene expression.   Target
sequences are chosen using a computer program which detects
sequences which are greater than 70% polypurine-polypyrimidine and
35   which are longer than 12 base pairs in length.   Of course, this can
be done by eye, however available software is much more efficient.
These sequences are then analyzed for protein binding and triplex
formation.   If triplex formation is detected, then an

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oligonucleotide conjugate e.g. acridine-oligonucleotide, is synthesized and its effect on protein binding tested.

The triplex forming oligonucleotides are chosen to be complementary to the polypurine strand of the target sequence in the promoter of the selected gene. As noted above, this requires, as a preliminary step, that a polypurine/polypyrimidine sequence exists in the promoter of the gene of interest. Once such a sequence is identified, a target sequence is selected in the gene promoter which is preferably from about 15 to about 20 bp long and about 80% polypurine/polypyrimidine. Then an oligonucleotide is synthesized which is complementary to the polypurine strand of the target. The potential for triplex formation can then be assessed by both parallel and anti-parallel oligonucleotides.

The oligonucleotide is complimentary to the polypurine strand of the target sequence and is thus, of necessity, largely polypyrimidine. This allows maximal chance of triplex formation. In some instances subtle base substitutions may be made in order to increase triplex affinity.

The preparation of the conjugates of the instant invention may be further exemplified by the following non-limiting example of the preparation of a preferred embodiment of the claimed invention.

EXAMPLE 1

The preparation of the acridine-linker molecule is carried out as follows. The initial step in the preparation of the acridine-linker molecule comprises the reaction of $H_2N(CH_2)_6OH$ with methoxy-2-dichloro-6,9-acridine.
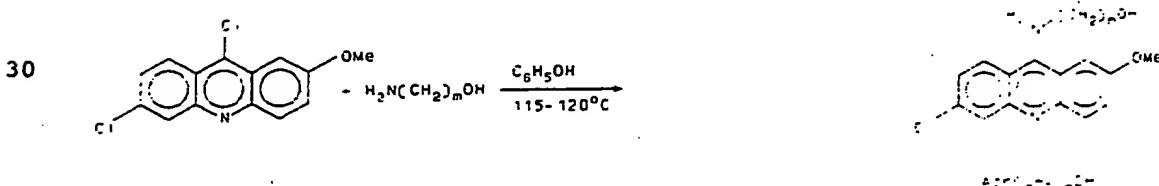

$H_2N(CH_2)_6OH$ (two equivalents) was added to methoxy-2-dichloro-6,9-acridine (two equivalents) in 2.5 g phenol at 80°C. The temperature was increased to 110°C and maintained at that level for one hour and 30 minutes. The progress of the reaction was followed by thin layer chromatography in solvent A (CH_2Cl_2/MeOH, 90:10)

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(acridine Rf 0.9, product Rf 0.15). When the reaction was complete, MeOH was added and the reaction was poured dropwise, with stirring, into NaOH at 0°C. The precipitate was washed with water until the pH was neutral. The yellow precipitate was crystallized in H_2O/MeOH (20:80). Complete removal of the phenol was documented by the DBPNC test. This acridine-linker molecule can be synthesized in large quantities and used for the synthesis of multiple target compounds.

EXAMPLE 2

The binding of the acridine-linker to the oligonucleotide is represented by the following scheme.
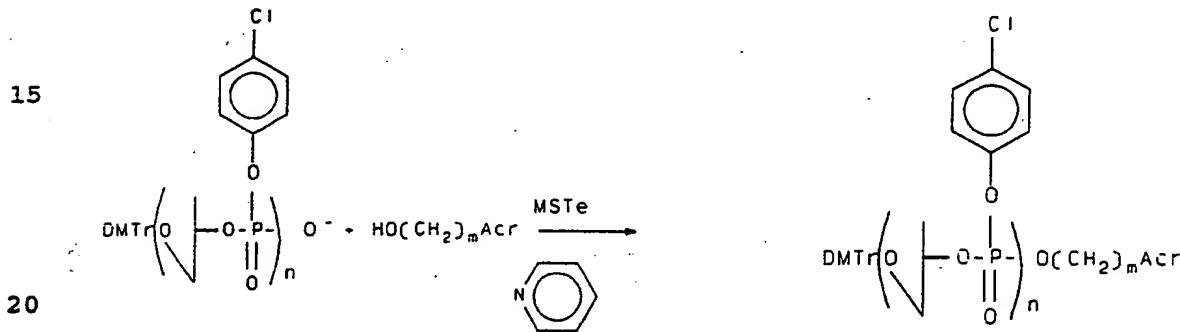
A solution of 5' phosphorylated nucleotide (one equivalent) was mixed with the modified acridine-linker molecule (1.3 equivalents) in anhydrous pyridine. Mesitylene sulfonyl trichloride (2.5 equivalents) was added at room temperature and the reaction was maintained at room temperature for 40 minutes. The excess reactants were destroyed by the addition of cold water (4°C) and the reaction mixture was extracted with chloroform since, due to the unprotected phosphate groups, the complexed oligonucleotide remains in the aqueous layer. The aqueous phase was reextracted with chloroform and dried. The acridine-oligonucleotide conjugate was purified by silica gel chromatography in buffer B (CH_2Cl_2/MeOH/pyridine 94:5.5:0.5) and eluted with water. Each compound was purified by reverse phase HPLC in TEAA and acetonitrile. The purified product is characterized by UV spectroscopy.

In an analogous manner, attachment to the 3' end may be accomplished as follows:

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EXAMPLE 3

The 6-chloro-9-(p-chlorophenoxy)-2 methoxy acridine is prepared as follows: 5 mM of 6,9-dichloro-2-methoxy acridine is mixed with 23 mM of p-chlorophenol and heated at 80°C for 4 hours. The
5 reaction mixture is poured into hot 5% aqueous NaOH and the crystals which form are washed with water to neutrality, dried and recrystallized from pyridine.

Ten uM of 3' alkyl amine linked to the targeted oligonucleotide is mixed with one uM of 6-chloro-9-(p-chlorophenoxy)-2 methoxy
10 acridine in pyridine. The reaction mixture is heated at 60°C overnight. After dilution with 1 ml of water the reaction product is extracted with chloroform. Purification may be carried out using reverse phase HPLC and/or gel filtration.

Once the DNA binding drug-oligonucleotide conjugates have been
15 synthesized, it is important to determine in vitro triplex formation prior to the use of such compounds for the specific inhibition of gene expression. The inventor has used polyacrylamide gel analysis of triplex formation to demonstrate that the triplex forming oligonucleotide can form both duplex and
20 triplex DNA structures. Similarly, DNAse 1 footprinting analysis is used to determine the relative sequence specificity of this binding. In vitro and in vivo experiments have demonstrated that, for example, the c-myc targeted conjugate binds specifically to its target sequence in vitro, prevents protein binding and inhibits
25 expression of the target gene. Moreover, the conjugate exhibits sequence specific binding intracellularly at concentrations as low as 0.02 uM.

In one embodiment of the instant invention employing the acridine-oligonucleotide conjugate, both duplex and triplex
30 formation by the c-myc binding site targeted conjugate occur at relatively low drug to target ratios. Although the addition of acridine to the 14 bp oligonucleotide results in only a slight change in molecular weight, the addition of the acridine moiety results in a detectable retardation in gel migration. This has
35 allowed the inventor to document the formation of duplex and triplex DNA structures in the presence of an increasing concentration of acridine-oligonucleotide conjugate.

Polyacrylamide gel analysis of the triplex formation demonstrates that the triplex forming oligonucleotides can form

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both duplex and triplex DNA structures. As shown in Figure 1, both
duplex and triplex formation by the c-myc targeted molecule occur
at relatively low drug:target ratios. The c-myc targeted conjugate
is comprised of acridine, a six methylene linking molecule, and a

5   27 bp G-rich triplex forming oligonucleotide. The G-rich 27 bp
oligonucleotide conjugate forms triplex DNA as shown in Figure 1,
while the C-rich complementary conjugate does not. Although the
addition of acridine to the 27 bp oligonucleotide results in only
a slight change in molecular weight, it is clear that the addition

10   of the acridine moiety results in an obvious detectable retardation
in gel migration. Thus, formation of duplex and triplex DNA
structures in the presence of increasing concentrations of
oligonucleotide complexed acridine is clear.

The sequence specificity of drug binding may be determined by

15   DNAse 1 footprint analysis as shown in Figure 2. The
oligonucleotide targeted conjugate demonstrates sequence
specificity, as shown by the DNAse 1 footprint pattern which is
obtained in the presence of targeted conjugate, as compared to the
control digestion. Control experiments demonstrate that this

20   sequence is not footprinted with acridine alone, or with the
triplex forming oligonucleotide alone. This indicates that the
attachment of acridine to the triplex forming oligonucleotide
results in a marked increase in the binding affinity of the
conjugate.

25   When triplex formation with the conjugates of the instant
invention is compared to that of oligonucleotide alone it is clear
that the addition of the DNA binding drug results in an increased
binding affinity. Gel mobility shift analysis was used to
determine the effect of conjugate triplex formation on protein

30   binding to the target sequence. The P1 flanking region DNA
fragment was incubated in an HeLa cell extract. Protein binding
was determined by polyacrylamide gel electrophoresis. In some
experiments triplex formation was allowed to occur prior to
incubation in the extract. As shown in Figure 3, the G-rich, but

35   not the C-rich conjugate is able to prevent protein binding to the
P1 flanking sequence. This is consistent with the footprint
analysis demonstrating that the G-rich, but not the C-rich
conjugate can form triplex DNA. This clearly indicates that

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triplex formation by the acridine-oligonucleotide conjugate prevents protein binding to the target sequence.

Until now, it has been impossible to study the specificity of drug-DNA interactions in whole cells. The development of polymerase chain reaction (PCR) based in vivo footprinting has obviated many of the problems with intracellular analysis of drug-DNA interactions. The inventor has used this to detect interaction of DNA binding drugs with specific DNA sequences in vivo (in whole cells). By amplifying the sequence of interest using PCR, the binding of the c-myc targeted acridine-oligonucleotide conjugate to its target sequence in the c-myc promoter can be analyzed. HL-60 promyelocytic leukemia cells in culture were treated with dimethylsulfate (DMS), lysed and the DNA treated with piperidine. There was clear evidence of c-myc targeted conjugate binding to the target sequence in the c-myc promoter in these cells at very low concentrations (0.02 uM). The acridine oligonucleotide binding was determined in several ways. Intracellular binding was documented by in vivo footprint analysis. This PCR based technique allowed detection of specific binding at concentrations as low as 0.2 um. In vitro binding was documented by gel retardation and DNAse I protection. The protection pattern is similar to that obtained with the DNAse 1 footprinting, in vitro.

The instant invention has considerable practical utility with regard to clinical research applications. Among the genes which the instant invention can be adapted to inhibit there may be mentioned cancer causing oncogenes, growth factor genes, viruses such as the AIDS virus, growth factors and growth factor receptors. For example, amenable target genes are the c-myc protooncogene which is overexpressed in almost all malignancies, the c-neu protooncogene, the HIV-LTR gene, beta-globin and the multiple drug resistance gene.

For example, c-myc overexpression appears to be absolutely necessary for the rapid cellular proliferation of HL-60 cells. As show in figure 4, 5 mM of acridine alone has little effect on thymidine incorporation by HL-60 cells. Similarly, the triplex forming oligonucleotide alone has little effect on DNA synthesis by these cells after 48 hours of exposure. The acridine-oligonucleotide conjugate, on the other hand (5 mM), resulted in 94% inhibition of DNA synthesis. This indicates that this c-myc

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targeted conjugate is an effective anti-proliferative agent. These results were confirmed by studying the effect of this conjugate on cell number. Once again, the oligonucleotide alone had only slight effect on inhibition of cellular proliferation as shown in Figure

5   5.   The c-myc targeted conjugate, however, demonstrated a concentration dependent inhibition of cellular proliferation. This indicates that the effect of this particular c-myc targeted conjugate may be specific for c-myc expression.

In order to document the effect of the conjugate on c-myc

10   expression, RNA was isolated from HL-60 cells at various times following drug addition. As shown in Figure 6, there is dramatic and early inhibition of c-myc expression by the G-rich, but not the C-rich conjugate. There is no inhibition of c-myc expression by the 3 bp substituted conjugate. This data suggests that the c-

15   myc promoter targeted conjugate is able to specifically inhibit expression of the c-myc protooncogene.

In vivo studies in mice with acridine-oligonucleotide conjugates synthesized with $3'-P^{32}$ labelled oligonucleotide were carried out as follows. 1×10^6 CPM were injected into the mouse

20   tailvein. Blood (10ul) was removed at 10 minute intervals for 1 hour. The half-life in blood was 30 minutes. Each animal was analyzed by Geiger counting at 0.1/2, 2, 4, 8, 12, 24, 48, 72, 96 hours. The whole animal half-life was 48 hours. This agreed with the low amount of radioactivity in the urine of the mice. Analysis

25   of the radioactivity remaining in the mice at 96 hours revealed that a substantial proportion of the conjugate was intact.

Experiments performed in rats demonstrated remarkable stability of the conjugates with a half life of about 24 to 36 hours. In contrast, the oligonucleotide itself only has a half life of about

30   15 to 30 minutes. The conjugates may be administered parenterally in liquid or solid form. Intravenous or tablet forms are suitable for administration of the instant invention in conjunction with pharmaceutically acceptable excipients or carriers. Dosages may range from about 1 to about 30 mg which may be administered as

35   needed. Other diseases which might be amenable to this approach include hemoglobinopathies, cardiac hypertrophy and restenosis of coronary arteries following angioplasty. The only limitation of the claimed invention is the sequence of the target promoter gene

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since it is necessary to identify a triplex forming sequence within
the promoter of the gene of interest.

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WHAT IS CLAIMED IS:

1. A sequence specific DNA binding molecule which comprises an oligonucleotide conjugate formed by the covalent attachment of a DNA binding drug to a triplex forming oligonucleotide.

2. The sequence specific DNA binding molecule of claim 1, wherein the DNA binding drug is selected from the group consisting of mithramycin, acridine, actinomycin D, doxorubicin, daunorubicin, mitomycin C, bleomycin, distamycin, netropsin and lexitropsin or derivatives thereof.

3. The sequence specific DNA binding molecule of claim 1 which is an acridine-oligonucleotide conjugate formed by the covalent attachment of acridine to a triplex forming oligonucleotide.

4. A method of preventing regulatory protein binding and transcription initiation in DNA comprising, delivering to said DNA a sequence specific binding molecule which comprises an oligonucleotide conjugate formed by the covalent attachment of a DNA binding drug to a triplex forming oligonucleotide.

5. The method according to claim 4, wherein said oligonucleotide conjugate is an acridine-oligonucleotide conjugate formed by the covalent attachment of acridine to a triplex forming oligonucleotide.

6. The method according to claim 4, wherein said conjugate is specific to the protein binding regions in the promoter of c-myc.

7. A method of modulating gene expression comprising administering to a mammal in need thereof a sequence specific binding molecule which comprises an acridine-oligonucleotide conjugate formed by the covalent attachment of acridine to a triplex forming oligonucleotide.

8. A method according to claim 4, wherein the gene is selected from the group consisting of the c-myc gene, beta-globin gene, dihydrofolate reductase gene, c-neu protooncongene, the multiple drug resistance gene and HIV-LTR gene.

9. A method according to claim 4, wherein the gene is the c-myc gene.

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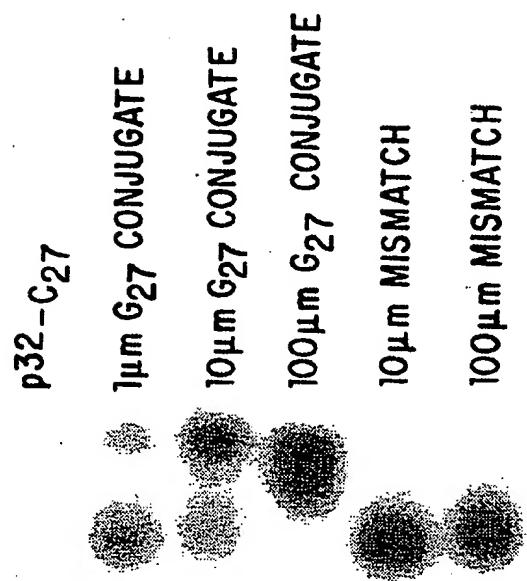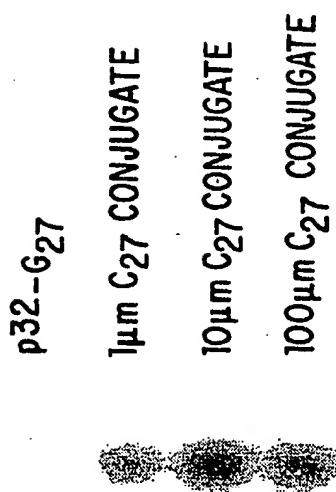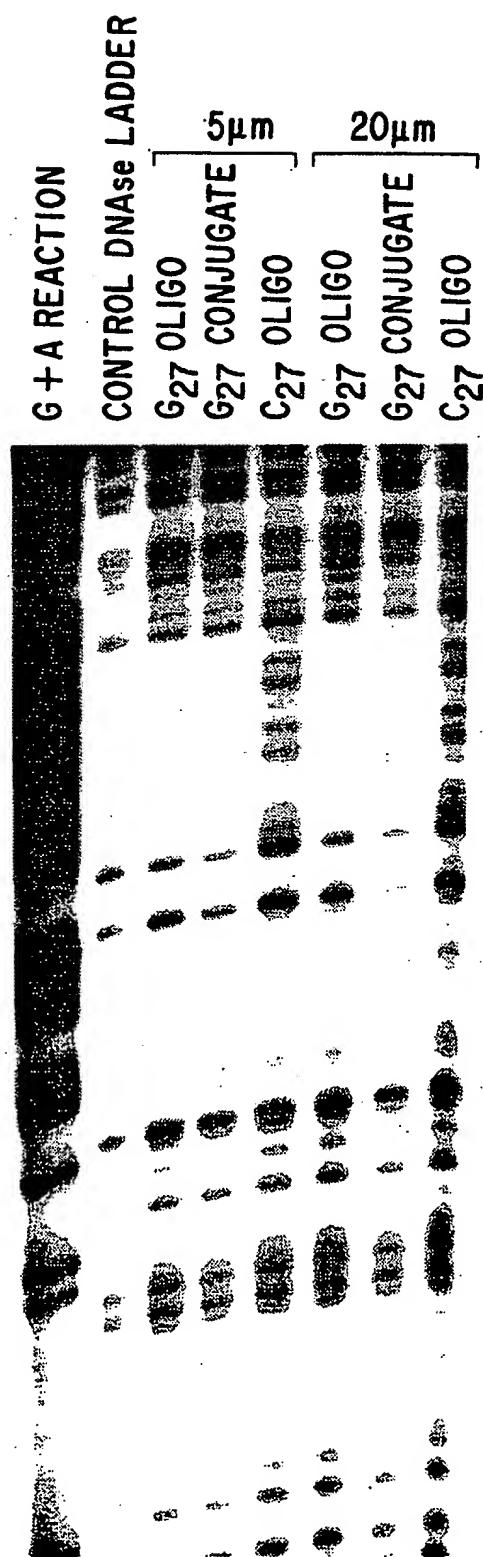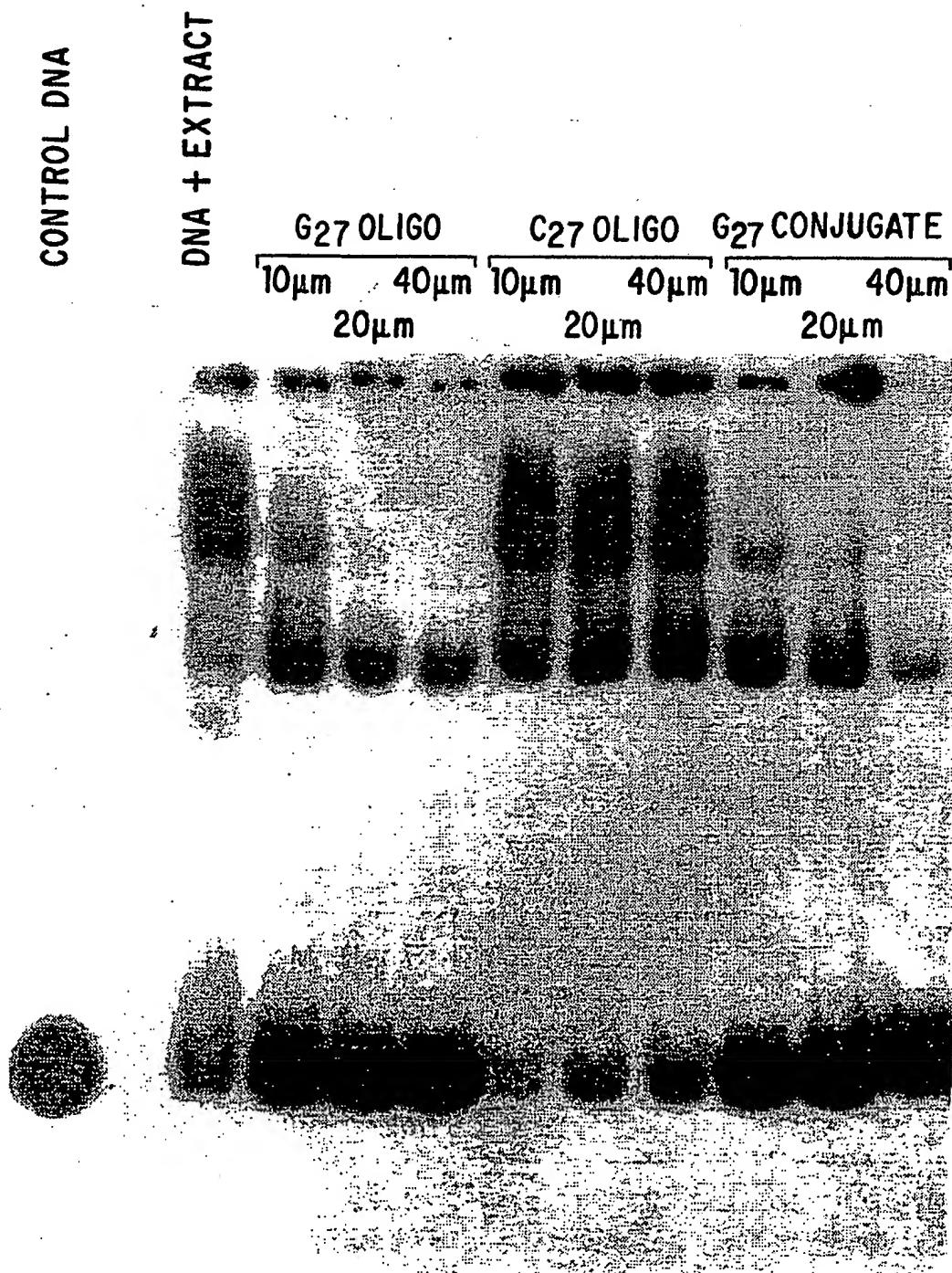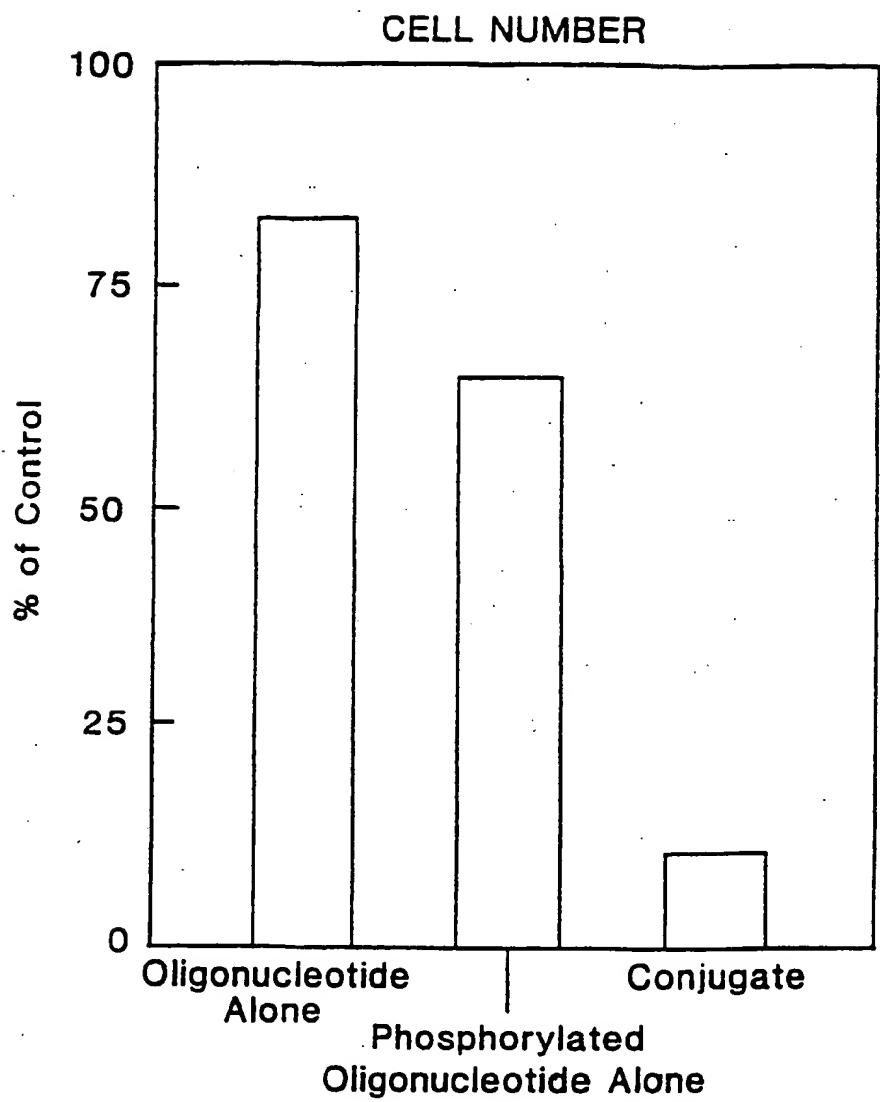
FIG. 1A

FIG. 1B

FIG. 2

FIG. 3

FIG. 4
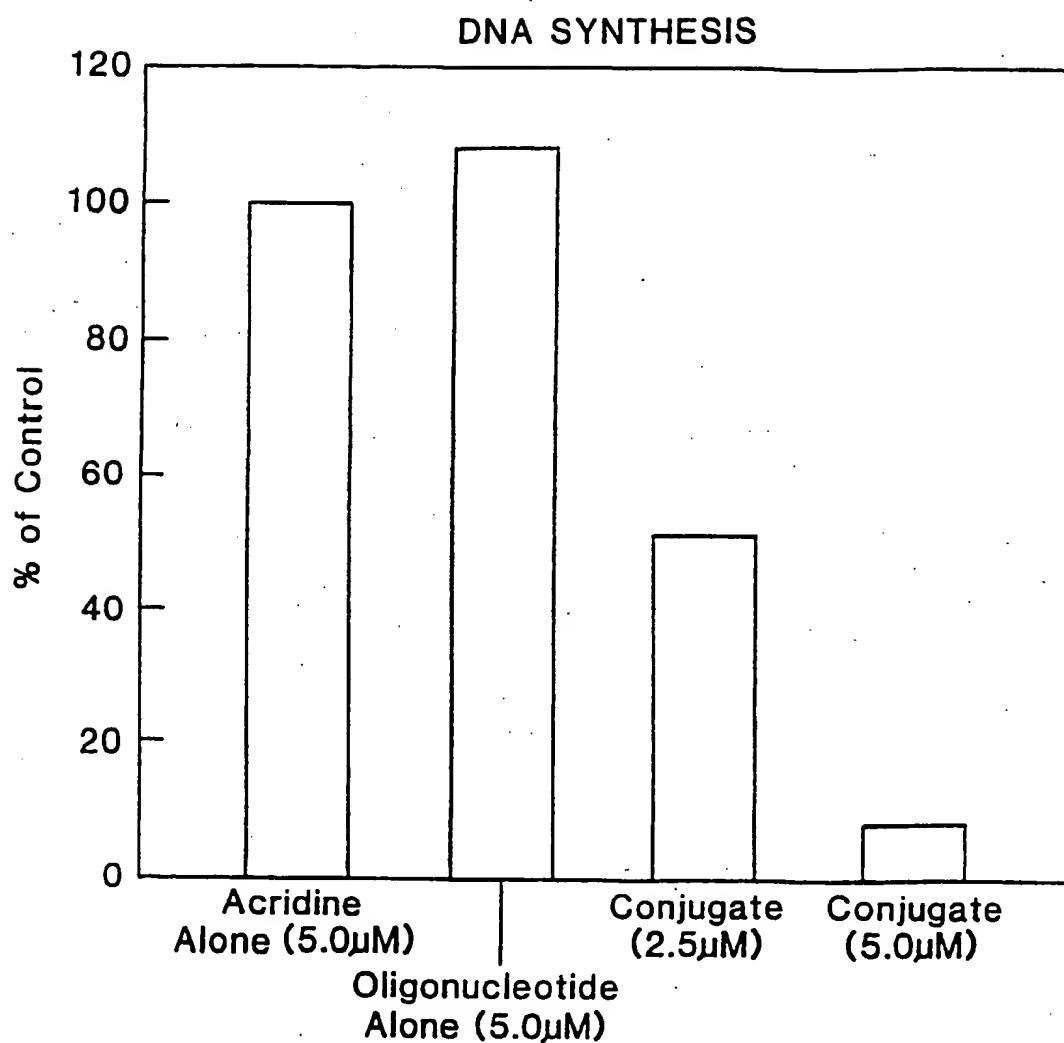
FIG.5
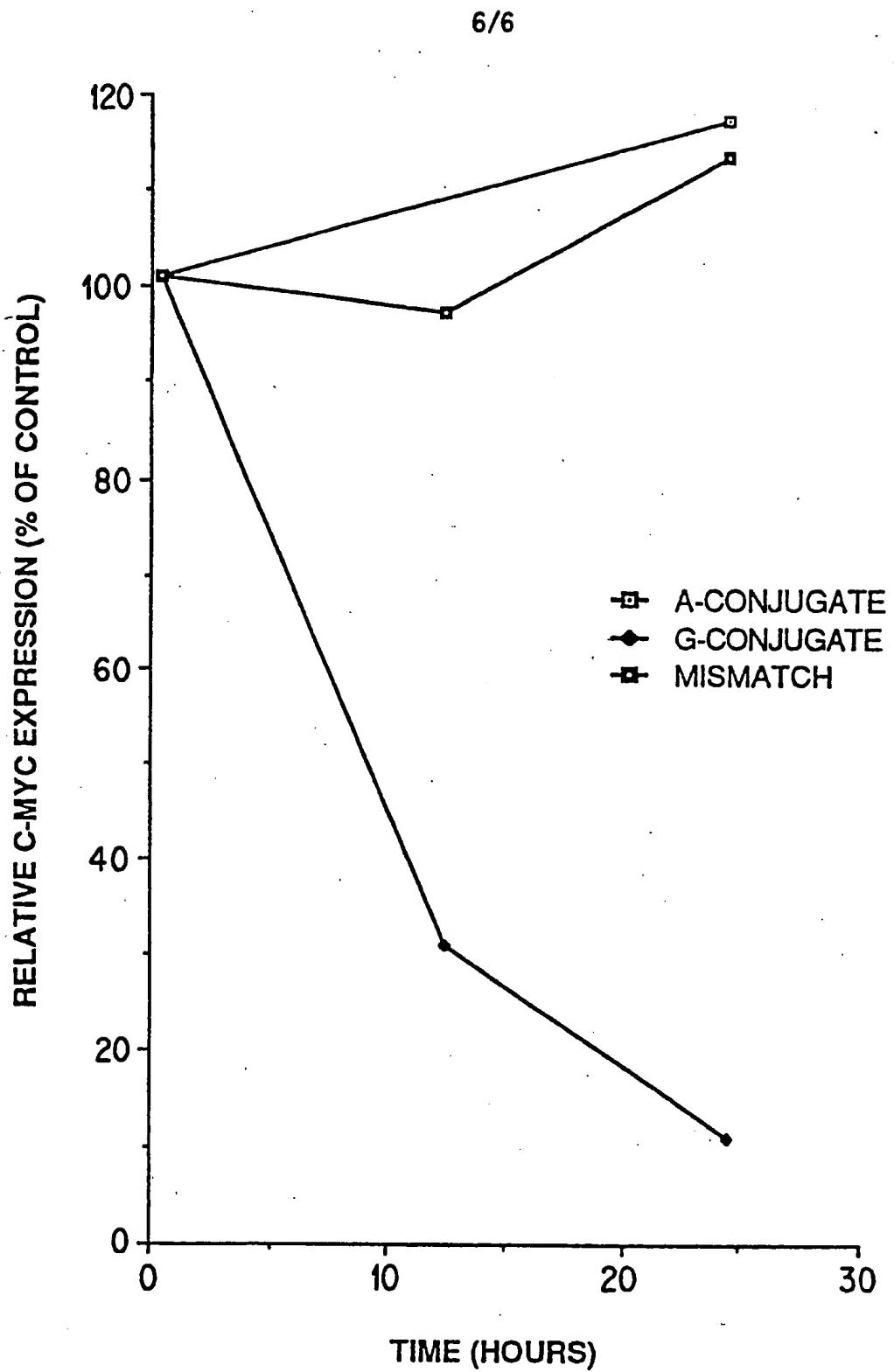
FIG.6

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07H 21/04; A61K 31/70; C12N 15/00
US CL :536/27; 514/44; 435/172.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/27; 514/44; 435/172.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Biosis, World Patent Index.
Search terms: triplex, triple hilix, promoter(s)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Science, Vol. 241, issued 22 July 1988, Cooney et al., "Site-specific oligonucleotide binding represses transcription of the human c-myc gene in vitro", pages 456-459. See entire article.	1-9
Y	Journal of Clinical Investigation, Vol. 83, issued June 1989, Ray et al., "Mithramycin blocks protein binding and function of the SV40 early promoter", pages 2003-2007. See entire article.	1-9
Y	Biochimica et Biophysica Acta, Vol. 418, issued 1976, Canellakis et al., "Diacridines: Bifunctional intercalators; I. Chemistry, physical chemistry and growth inhibitory properties", pages 277-289. See entire article.	3,5

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

* Special categories of cited documents:

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Date of the actual completion of the international search	Date of mailing of the international search report
11 August 1992	19 AUG 1992
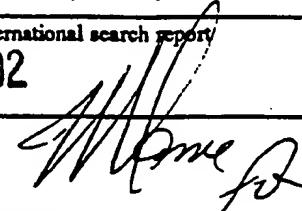
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer PHILIP W. CARTER
Facsimile No. NOT APPLICABLE	Telephone No. (703) 308-0196